Angiogenic changes in a new mouse model for hepatocellular carcinoma

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BACKGROUND

The increasing incidence of hepatocellular carcinoma in Western countries has led to an expanding interest in this field. A vast need of experimental models that mimic the natural pathogenesis of hepatocellular carcinoma in a short time period is present. The goal of our study was (1) to develop an efficient mouse model for hepatocellular carcinoma research, (2) to assess time-dependent changes angiogenic pathways and (3) to investigate tumour growth and neo-vascularisation using state-of-the-art imaging techniques.

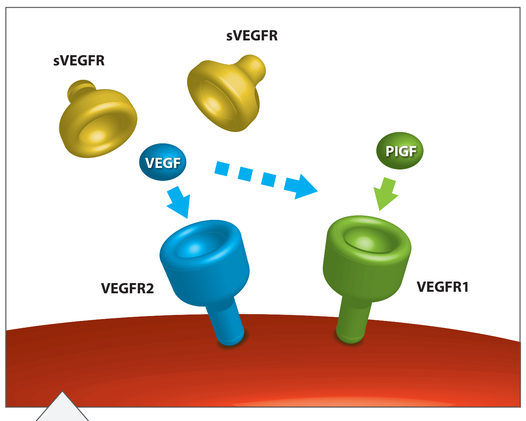
METHODS

5-week-old male mice received weekly intraperitoneal injections with N-nitrosodiethylamine (DEN) (35 mg/kg bodyweight) and samples were taken at several time points. Histology, ELISA and immunohistochemical stainings were used to identify the HCC-lesions and to quantify angiogenic factors VEGF and PlGF; and their receptors. HCC livers (25W) were perfused with Batson's n°17 solution to produce vascular casts (arterial and venous). A state-of-the-art multimodal microPET/CT was used for in vivo detection for 3D-reconstruction of the vascular casts.

RESULTS

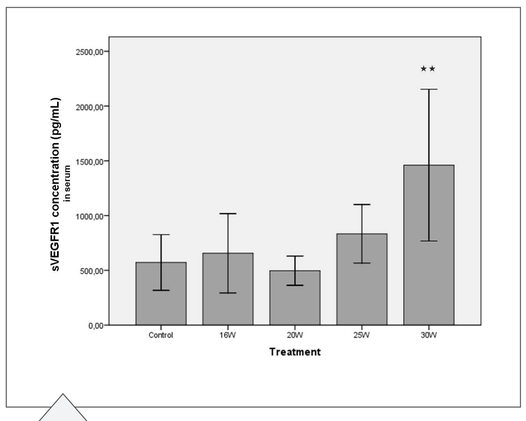
After 16W of DEN-injections a mild fibrosis (F1-F2) and dysplastic lesions appear, resulting in a pre-malignant environment. An increase of angiogenic factors VEGF and PlGF takes place, but not explicit enough to induce an increase in endothelial cells, which were upregulated after 20W. After 25W of DEN-injections, the dysplastic lesions have progressed to vascularised exophytic tumours which are macroscopically visible and give rise to a further increase in angiogenic factors, activating the angiogenic pathway and leading to the formation of new blood vessels.

The vascular casts of HCC-livers clearly revealed the chaotic pattern and hierarchically disorganisation of tumour induced blood vessels. Arteries formed a circumferential mantle around the hepatic tumours, while the central tumour regions showed a lower arterial density. Electron microscopy revealed several angiogenic spots, with mostly sprouting angiogenesis, furthermore intussusceptive angiogenesis was also seen.

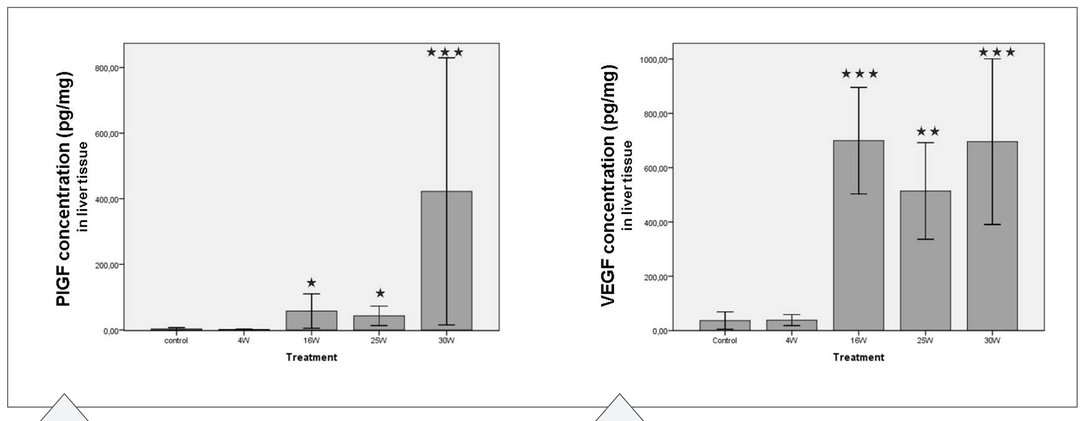


Angiogenesis

Up till now VEGF, was one of the key targets in tumour induced angiogenesis, but recently PLGF gained interest because its association with pathological conditions. Both VEGF and PLGF can bind to VEGFR1, though only the latter can induce signal transduction leading to neo-vascularisation and arteriogenesis. Soluble VEGF receptors are known to capture VEGF without inducing signal transduction and therefore serve as decoy receptors.

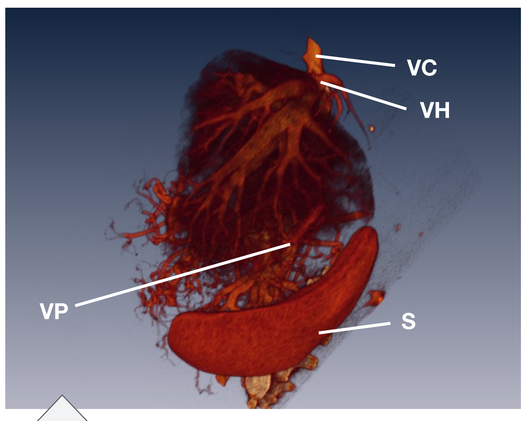


Soluble vascular endothelial growth factor receptor-1 (sVEGFR1), a naturally occurring soluble form of the VEGF receptor1, is an important negative counterpart of the VEGF signalling pathway. Therefore, we measured the serum levels of sVEGFR1. There was a slight increase of sVEGFR1 at 30W DEN treatment, but no significant difference was seen between controls and 25W DEN

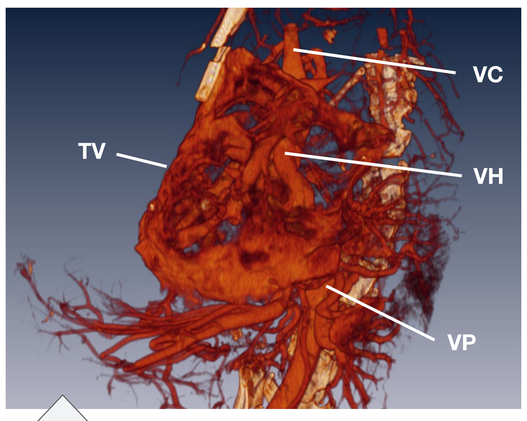


protein levels of PlGF in liver tissue, the vast up-regulation of PlGF in liver tissue after 30W DEN veils the 2.4x increase at 25W

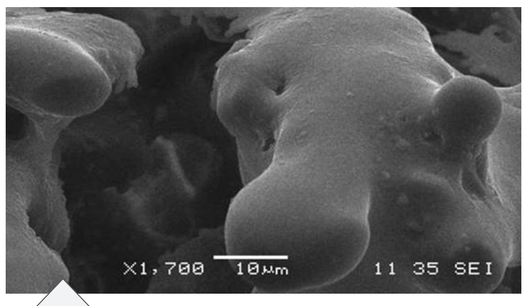
protein levels of VEGF in liver tissue. Asterisks (*) represent the significant P-values of the control group compared to DEN-groups (* = p<0,05; ** = p<0,01 and *** = p < 0,001). These data support the theory that PLGF plays an important role in the pathogenesis of HCC.



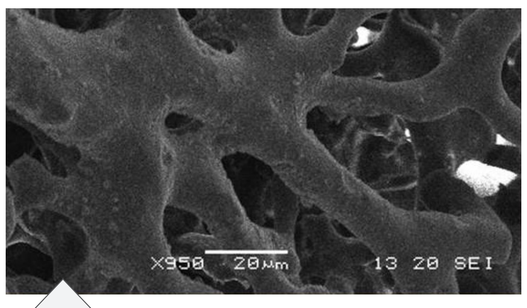
Venous cast control liver



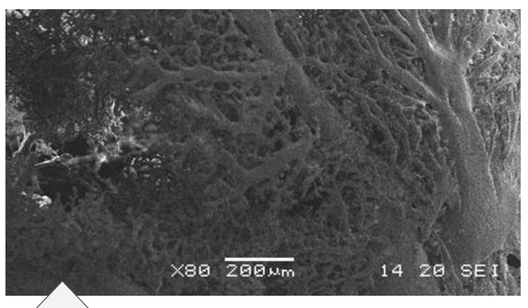
Venous cast HCC liver



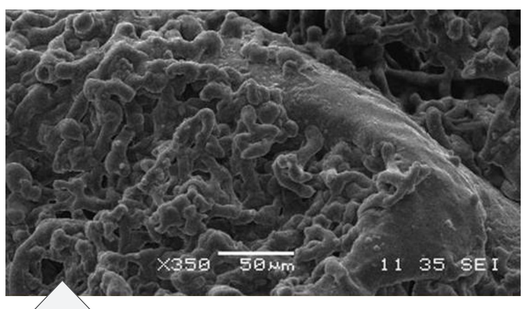
Sprouting angiogenesis



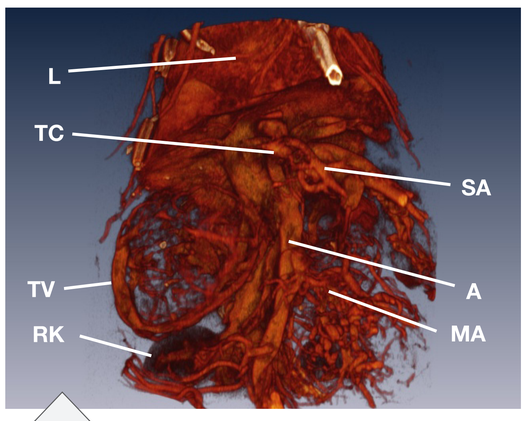
Intussusceptive angiogenesis



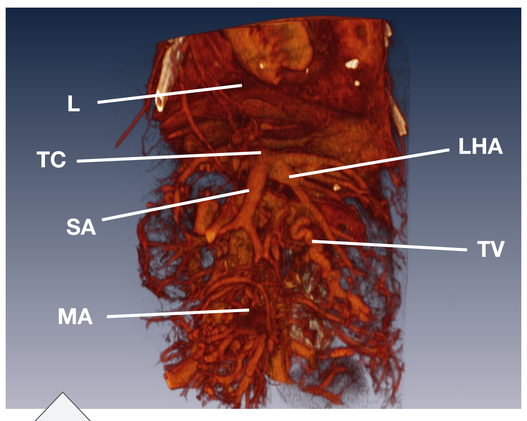
Arterial mantle



Microvasculature



Arterial cast HCC liver (right lobe)



Arterial cast HCC liver (left lobe)

A = Aorta; **L** = Lungs; **(L)HA** = (left) hepatic artery; **MA** = mesenteric artery; **RK** = right kidney; **S** = spleen; **SA** = splenic artery; **TC** = truncus coeliacus; **TV** = tumour vessels; **VC** = vena cava; **VP** = vena porta; **VH** = vena hepatica

CONCLUSION

While most DEN-induced models take at least one year to develop tumours, weekly injections with DEN give rise to tumour occurrence after 25W. The well vascularised orthotopic tumours are a representative model for HCC and can serve as an excellent platform for the development of new therapeutic targets. The histological and serological increase of angiogenic factors (PlGF & VEGF), clearly give rise to new blood vessel formation, confirmed by endothelial cell quantification and CT-reconstructions of vascular casts.

High levels of VEGF can predict vascular invasion of HCC and correlated with poor prognosis [1-2]. Elevated VEGF is also a marker for poor response to locoregional treatment and is correlated with early recurrence [3-4]. PlGF levels are known to be elevated in a variety of cancers and is associated with poor prognosis in HCC [5-6]. The up-regulation of PlGF in this DEN-model supports the theory that PlGF plays an essential role in the angiogenesis of HCC.

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